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Peroxisome Assembly in Yeast

MARTEN VEENHUIS,* JAN A.K.W. KIEL, AND IDA J. VAN DER KLEI

Eukaryotic Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, NL-9750 AA Haren, The Netherlands

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ABSTRACT Peroxisomes are essential organelles that may be involved in various functions, dependent on organism, cell type, developmental stage of the cell, and the environment. Until recently, peroxisomes were viewed as a class of static organelles that developed by growth and fission from pre-existing organelles. Recent observations have challenged this view by providing evidence that peroxisomes may be part of the endomembrane system and constitute a highly dynamic population of organelles that arises and is removed upon environmental demands. Additionally, evidence is now accumulating that peroxisomes may arise by alternative methods. This review summarizes relevant recent data on this subject. In addition, the progress in the understanding of the principles of the peroxisomal matrix protein import machinery is discussed. *Microsc. Res. Tech.* 61:139–150, 2003. © 2003 Wiley-Liss, Inc.

INTRODUCTION

Microbodies (peroxisomes, glyoxysomes, and glycosomes) are morphologically simple organelles. They consist of a single membrane that encloses a proteinaceous matrix and measure up to 1 μm in diameter. Despite their simple morphology, the functional diversity of these organelles is unprecedented and varies with the organism in which they occur. Thus, microbodies may be involved in such distinct functions as carbon catabolism in fungi, biosynthetic processes (e.g., synthesis of amino acids and penicillin in fungi, and of cholesterol in man), photorespiration in plants, or glycolysis in Trypanosomes (Borst, 1989; Van den Bosch et al., 1992; Veenhuis and Harder, 1988). Although many of the players that are involved in peroxisome biogenesis have now been identified, our understanding of the molecular principles of assembly and function of the organelle is still limited. So far, the data lend support to the view that the strategies that the cell uses to mediate peroxisome development may, at least in part, differ between various organisms. Here, we will highlight recent observations on fungal peroxisomes and discuss the current working models on the biogenesis of these intriguing organelles.

PEROXISOME PROLIFERATION

A characteristic feature of yeast peroxisomes is that they are inducible. The organelles rapidly develop during adaptation of cells to a new environment that requires one or more peroxisomal enzymes for growth. Growth substrates known to induce peroxisomes in various yeasts include alkanes, oleic acid, methanol, D-amino acids, purines, and primary amines (reviewed by Veenhuis and Harder, 1988). Remarkably, the number and volume fraction of peroxisomes is not prescribed by the amount of protein that is accommodated but, instead, appears to be predominantly determined by the growth conditions, i.e., the choice of the substrate, the growth rate, and the oxygen concentration (Veenhuis and Harder, 1988). Overproduction of specific matrix proteins in WT cells made it clear that the

maximal protein storage capacity of peroxisomes is normally not used (Distel et al., 1988; Godecke et al., 1989). Even in cells that contain identical sets of peroxisomal matrix proteins, the size and number of the organelles may strongly differ. This was elegantly demonstrated in glucose-limited continuous cultures of *Hansenula polymorpha*. When grown at high dilution rates, *H. polymorpha* cells generally contained a single large peroxisome that harbored the enzymes alcohol oxidase (AO), dihydroxyacetone synthase (DHAS), and catalase (CAT). At low dilution rates, however, several smaller organelles were present of identical protein composition (Fig. 1). Also, in *H. polymorpha* *Pim* mutants, in which a major portion of the peroxisomal matrix proteins resides in the cytosol, small peroxisomes were present at numbers equal or even exceeding those of WT cells (Waterham et al., 1992b). Taken together, these data leave little doubt that it is not the matrix protein levels but rather the environmental conditions that prescribe the ultimate number and size of peroxisomes, and, thus, control the multiplication of the organelle. So far, the highest peroxisome induction rates have been encountered in methylotrophic yeast species (e.g., *Candida boidinii*, *Hansenula polymorpha*, *Pichia pastoris*). When *H. polymorpha* is grown in methanol-limited chemostat cultures at low dilution rates, up to 80% of the total cytoplasmic volume of the cells may be occupied by peroxisomes (Fig. 2). Remarkably, these organelles display a crystalline substructure due to the crystallization of AO protein (Veenhuis et al., 1978).

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*Correspondence to: Prof. Dr. M. Veenhuis, Eukaryotic Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, P.O. Box 14, NL-9750 AA Haren, The Netherlands.

E-mail: M.Veenhuis@biol.rug.nl

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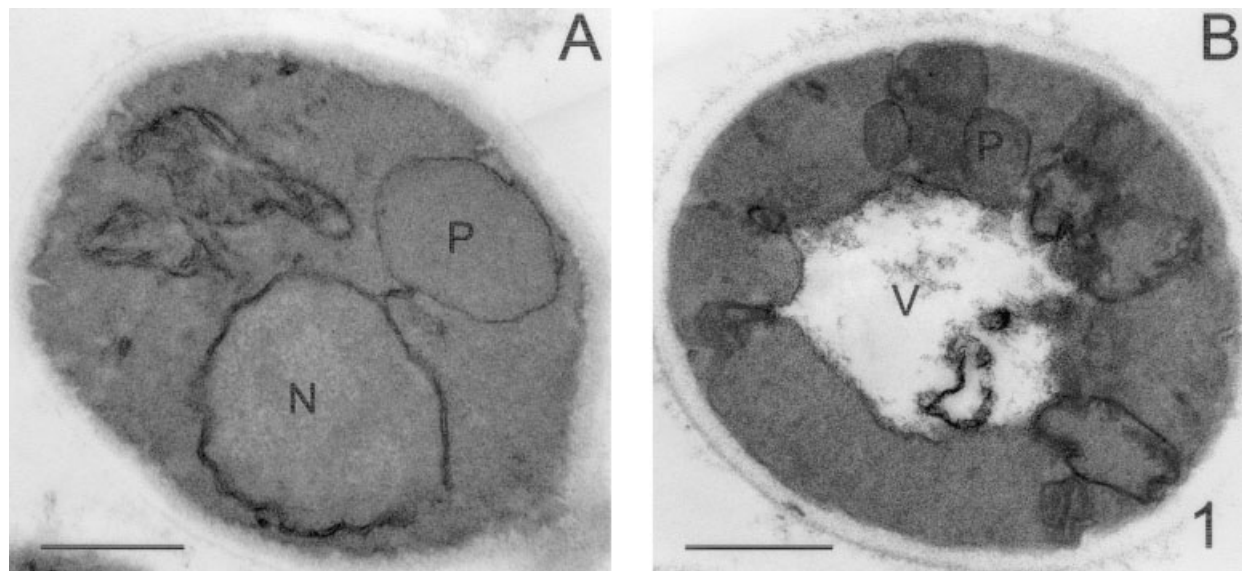


Fig. 1. Comparison of the size and number of peroxisomes in cells, grown in glucose-limited chemostats at different dilution rates. **A:** $D = 0.18 \text{ h}^{-1}$. **B:** $D = 0.05 \text{ h}^{-1}$. (For all figures, electron micrographs are taken of WT *H. polymorpha* cells, fixed with KMnO_4 unless otherwise stated. Abbreviations: N, nucleus; P, peroxisome; V, vacuole. Scale bars = $0.5 \mu\text{m}$.)

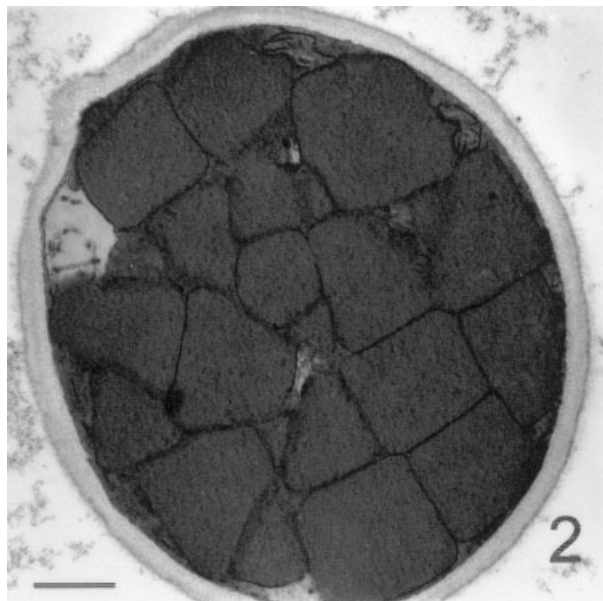


Fig. 2. Typical example of a cell grown in a methanol-limited chemostat ($D = 0.1 \text{ h}^{-1}$), crowded with peroxisomes.

Kinetics experiments, using *H. polymorpha* cells, revealed an unexpected characteristic of their peroxisomes, namely that these organelles are only temporally matrix protein import-competent. Small peroxisomes grow because of the incorporation of proteins and lipids. At a certain size, determined by the growth conditions (see below), growth ceases and one, or infrequently few, new organelles are formed by fission from the mature one, which in turn start to grow during prolonged cultivation (Fig. 3A,B). Apparently, these

small organelles have “inherited” the capacity to grow from the mature parent, leaving this organelle as an “enzyme bag” that is no longer capable of protein uptake but remains physiologically active (Veenhuis et al., 1989; Waterham et al., 1992a). The mechanisms that control peroxisome maturation and proliferation are largely unknown. However, studies on the function of the peroxisomal membrane protein Pex11p indicate that this protein might play a role in organelle multiplication. Overexpression of Pex11p results in proliferation of peroxisomes while its absence results in the formation of giant peroxisomes, causing problems to distribute peroxisomes over mother and daughter cells during cell division (Erdmann and Blobel, 1995; Marshall et al., 1995). Indeed, the group of Goodman showed that Pex11p induction *in vivo* resulted in fragmentation of large peroxisomes into smaller organelles (Marshall et al., 1996). The authors suggested that the oligomeric state of Pex11p could play a role in peroxisome maturation as the protein was found as monomer in small, newly formed organelles and as dimer in mature ones. Recently, however, a direct role for Pex11p in β -oxidation in *Saccharomyces cerevisiae* was proposed. Van Roermund and coworkers (2000) concluded that Pex11p is required to transport medium chain fatty acids into peroxisomes. In their view, its effect on peroxisome proliferation is only indirect resulting from an increased β -oxidation. Although such a scenario may explain the results obtained in *S. cerevisiae*, the proliferation effect upon Pex11p overproduction observed in, e.g., Trypanosomes that mainly play a role in glycolysis (Lorenz et al., 1998), cannot be reconciled with an exclusive role for Pex11p in fatty acid transport. Clearly, more research is required to unravel the mechanisms controlling peroxisome maturation/proliferation.

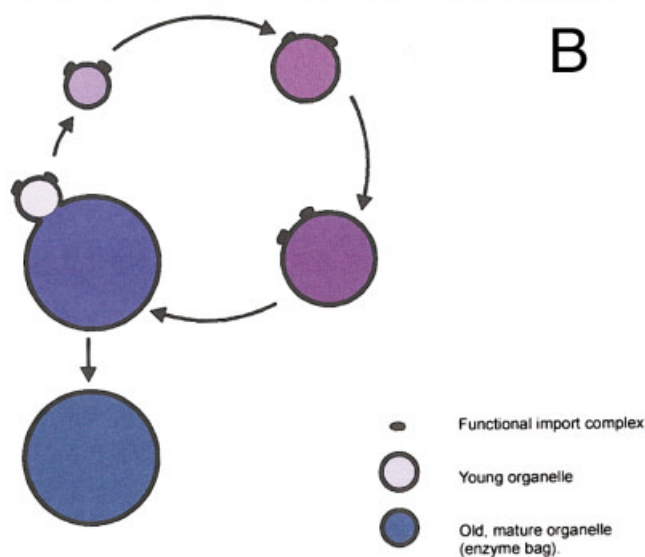
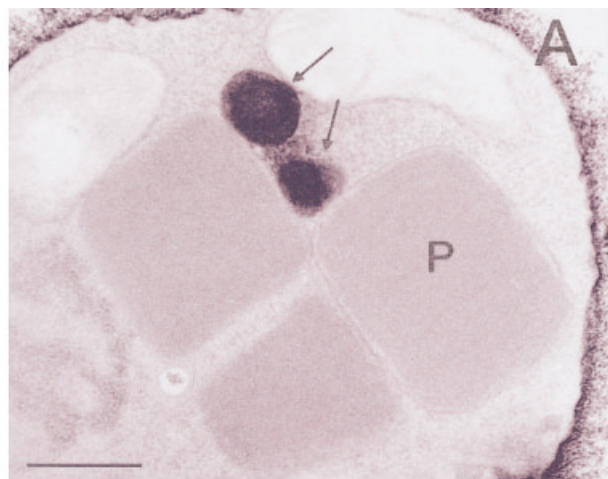


Fig. 3. **A:** Detail of a cell, to demonstrate the temporally matrix protein import competence of mature peroxisomes. Cells were pre-grown on methanol/ammonium sulfate, and subsequently switched to a new growth environment containing methylamine as the sole nitrogen source, to induce peroxisomal amine oxidase, key enzyme of amine metabolism. Two hours after the shift, the cells were analyzed for the localization of amine oxidase activity (CeCl_3 + methylamine). As evident in the micrograph, only the two small organelles (arrows) display enzyme activity while staining is absent in the large mature organelles (glutaraldehyde/ OsO_4). **B:** Hypothetical model to explain the temporary matrix protein import capacity of *H. polymorpha* peroxisomes. This model predicts that peroxisomes grow by the uptake of proteins and lipids. Matrix protein import is facilitated by distinct protein complexes on the peroxisomal membrane that are donated to small organelles that bud off from this organelle upon its maturation. This way, the mature organelle has lost the capacity to incorporate matrix protein and now serves as an enzyme bag to fulfill specific metabolic functions prescribed by the growth environment. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Peroxisomes that have become redundant for growth, i.e., upon a shift of cells from peroxisome inducing (e.g., methanol) to peroxisome-repressing (e.g., glucose) conditions, are degraded by a selective process

designated pexophagy (reviewed by Bellu and Kiel, 2003; see this issue). Studies in *H. polymorpha* have suggested that in particular mature organelles be degraded leaving the small, import-competent ones unaffected. Recently, we showed that a protein involved in the biogenesis of peroxisomes, the peroxin Pex14p, is also involved in pexophagy and may act as a molecular switch that discriminates between the import competence and incompetence of individual organelles (Bellu et al., 2001). The physiological advantage of this mechanism is immediately clear because it allows the cells to rapidly adapt to new growth environments that may require one or more new peroxisomal functions. Taken together, these data stress the flexibility of peroxisomes; their number, function, and volume fraction is rapidly adapted to prevailing environmental conditions.

PEROXISOME BIOGENESIS

Genes Involved in Peroxisome Biogenesis

Molecular approaches to identify proteins essential for peroxisome biogenesis (peroxins) became feasible in the early 1990s when viable yeast mutants were isolated that were defective in peroxisome development and function (collectively called *pex* mutants; see Distel et al. (1996) for the unified nomenclature). Yeast *pex* mutants have lost the capacity to grow on carbon sources (e.g., fatty acids, methanol)—but not nitrogen sources (e.g., primary amines)—that are metabolized by peroxisomal enzymes. Corresponding genes were cloned by functional complementation of these mutants, using genomic or cDNA libraries. At present, 23 different *PEX* genes from various yeasts are now identified (*H. polymorpha*, *S. cerevisiae*, *P. pastoris*, and *Yarrowia lipolytica*; (reviewed by Subramani, 1998; for a recent listing see <http://www.peroxisome.org/>). Most *PEX* gene products (termed peroxins) that have been identified so far are thought to play a role in matrix protein import and are discussed below.

Matrix Protein Import Signals

Peroxisomal matrix proteins are encoded by nuclear genes and are synthesized in the cytosol on free ribosomes (Lazarow and Fujiki, 1985). So far, two peroxisomal targeting signals have been characterized that mediate sorting of the protein to peroxisomes (De Hoop and AB, 1992; Rachubinski and Subramani, 1995; Subramani, 1998). Most proteins contain a PTS1 signal, a tripeptide that is located at the extreme C-terminus of matrix proteins. The PTS1 consensus sequence is $-\text{SKL}.\text{COOH}$, but various (conserved) variants of this motif are allowed (Gould et al., 1989; Lametschwandner et al., 1998). The PTS2 is located at the N-terminus and consists of the consensus $(\text{R/K})-(\text{L/V/I})-\text{X}_5-(\text{H/Q})-(\text{L/A})$ (Swinkels et al., 1991). Most likely other, possibly internal, signals also exist for a subset of specific proteins, e.g., malate synthase and acyl CoA synthase (Bruinenberg et al. 1990; Karpichev and Small, 2000; Kragler et al., 1993; Small et al., 1988). However, the exact amino acid sequences comprising these signals have yet to be identified.

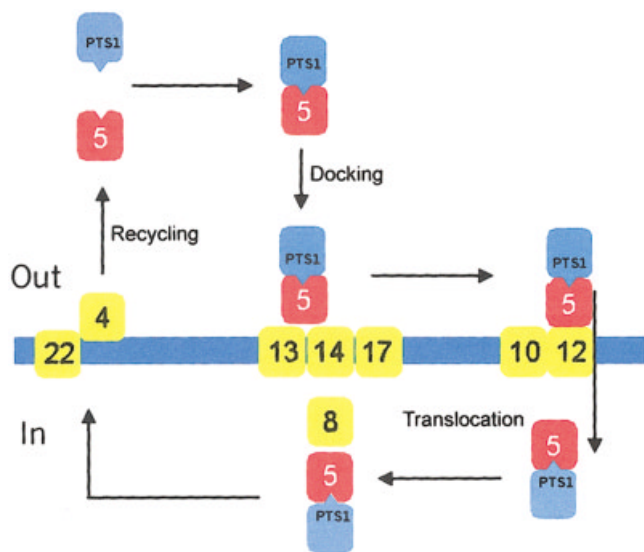


Fig. 4. Schematic representation of the extended shuttle model for PTS1 matrix protein import in *H. polymorpha*. Precursor PTS1 proteins, synthesized in the cytosol, bind to the PTS1 receptor (Pex5p) and are transported to a peroxisomal docking site, consisting of Pex13p, Pex14p, and Pex17p. A complex of two ring finger proteins (Pex10p, Pex12p) that also may contain Pex2p may mediate translocation of the Pex5p.cargo complex. After translocation, dissociation of the Pex5p.cargo complex takes place, possibly mediated by Pex8p. Recycling of Pex5p to the cytosol requires the function of at least Pex4p. Whether the import and export of Pex5p proceeds via the same gate, which in that case is composed of a large protein complex, or requires different pores, is totally unknown. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

PTS1 Protein Import: 1. Receptor Binding to Matrix Proteins

The initial data on the location of the PTS1-receptor, Pex5p, in different organisms were conflicting and varied with the organisms and/or the experimental approaches used. The reported locations varied from exclusively associated with the peroxisomal membrane, solely in the cytosol or the peroxisomal matrix to a dual location in both the cytosol and the organellar matrix (Dodt et al., 1995; Elgersma et al., 1996a; Gould et al., 1996; Szilard et al., 1995; Terlecky et al., 1995; Van der Klei et al., 1995; Wiemer et al., 1995). This has led to different models for the mechanisms of PTS1 protein import. The current view held by most researchers, except for *Y. lipolytica* (Szilard et al. 1995), is that the bulk of Pex5p is present in the cytosol in conjunction with a minor portion that is associated with peroxisomes, bound to the peroxisomal membrane or present in the organellar matrix (Dodt and Gould, 1996; Subramani, 1998).

In *H. polymorpha*, Pex5p is localized in the cytosol and in the peroxisomal matrix; the putative membrane-bound portion of the protein is invariably below the limit of detection (Van der Klei et al., 1995, 1998). These observations have led to our current model that predicts that *H. polymorpha* Pex5p functions as a cycling receptor between the cytosol and the peroxisomal matrix (Van der Klei and Veenhuis, 1996; Fig. 4). This would implicate that a protein export mechanism must

exist for peroxisomes. Very recently, results have been presented by Subramani and co-workers that prove this "extended shuttle" mechanism for PTS1 protein import in human peroxisomes (Dammai and Subramani, 2001).

The function of Pex5p in PTS1 import has been studied in detail. Several authors have shown that the tetratricopeptide repeats (TPR), localized in the C-terminal two-thirds of Pex5p, bind the PTS1 (Brocard et al., 1994; Fransen et al., 1995; Szilard and Rachubinski, 2000; Terlecky et al., 1995). In detail, insight in how the PTS1 signal of a matrix protein can bind the TPR domains came from the 3-dimensional structure of the C-terminus of human Pex5p (Gatto et al., 2000) as well as from mutational analysis of *S. cerevisiae* Pex5p (Klein et al., 2001). Furthermore, Lametschwandtner et al. (1998) showed that additional targeting information could be present in the residues preceding the PTS1 in matrix proteins, which most probably modulate the strength of the interaction of the cargo protein with Pex5p.

PTS1 Protein Import: 2. Receptor-Cargo Docking to the Peroxisomal Membrane

Upon binding a PTS1, the receptor-cargo complex interacts at a putative docking site on the peroxisomal membrane en route to the peroxisomal matrix. This model requires that the affinity of Pex5p for the docking site increases upon binding of a PTS1 in order to prevent competition for the docking site between soluble and cargo-bound Pex5p. Indeed, overexpression of *PEX5* in *H. polymorpha* cells does not interfere with PTS1 protein import (van der Klei et al. 1995). Peroxins that are believed to participate in Pex5p/cargo docking include Pex13p, which contains a Src homology 3 (SH3) domain (Barnett et al. 2000; Elgersma et al., 1996a; Erdmann and Blobel, 1996; Gould et al., 1996), the coiled-coil protein Pex14p (Albertini et al., 1997; Brocard et al., 1997; Komori et al., 1997) and Pex17p (Huhse et al., 1998). Pex5p has been shown to directly interact with Pex13p (Urquhart et al., 2000) and Pex14p (Schliebs et al., 1999). Next to this, two hybrid analysis revealed that Pex14p interacts also with Pex13p and Pex17p (for reviews see Erdmann et al., 1997; Subramani, 1998). In the N-terminus of human Pex5p, a pentapeptide repeat motif (W-x-[E,D,Q,A,S]-[E,D,Q]-[F,Y]) that is conserved in other Pex5p's was found to bind to the N-terminus of human Pex14p (Schliebs et al., 1999). Also in its N-terminus, Pex14p contains a classical SH3 binding motif, PxxP, to which the SH3 domain of Pex13p binds. Although Pex5p does not contain such a PxxP motif, it has an alpha helical element in its N-terminus that interacts with the SH3 domain of Pex13p in an unconventional, non-PxxP-related manner (Barnett et al., 2000).

How Pex17p binds Pex14p had not been established yet. *S. cerevisiae* Pex17p is a peroxisomal membrane protein that is involved in matrix protein import (Huhse et al., 1998). Surprisingly, a *P. pastoris* pex17 mutant also partly mislocalizes peroxisomal membrane proteins. Furthermore, *P. pastoris* Pex17p has been shown not only to interact with Pex14p, but also with Pex19p, a peroxin that is important for the insertion of peroxisomal membrane proteins, possibly as a receptor for the peroxisomal targeting signal of membrane pro-

teins (mPTS). Hence, Pex17p may be a component of different subcomplexes of peroxins that are involved in both matrix protein import as well as biogenesis of the peroxisomal membrane (Snyder et al., 1999).

Although the two-hybrid approach identified the putative docking proteins for the PTS1 receptor, Pex5p was also shown to bind to peroxins in an indirect way that depended on the function of Pex14p (Girzalsky et al., 1999; Huhse et al., 1998). Therefore, two-hybrid results have to be treated with care and additional biochemical/biophysical experiments are needed to investigate the nature and properties of the binding between the various peroxins and possible other protein components. A first example of a biophysical study is the research of Schliebs and co-workers (1999), who analyzed the interaction of the N-terminal fragment of human Pex14p with human Pex5p by surface plasmon resonance spectroscopy. Recent studies of the same group, which also included fluorescence spectroscopic analyses, revealed that the conserved pentapeptide repeats in the N-terminus of Pex5p bind with very high affinity (dissociation constant in the low nanomolar range) to the N-terminus of Pex14p (Saidowsky et al., 2001). Surprisingly, this observation is not in line with the finding that in most organisms studied so far only minor amounts of Pex5p are bound to the peroxisomal membrane. Apparently, additional components (e.g., peroxins, PTS1 cargo proteins), other domains of Pex14p or the recently reported phosphorylation of Pex14p (Johnson et al., 2001; Komori et al., 1999) may influence the Pex5p-Pex14p binding event. Schliebs et al. (1999) furthermore showed that one Pex5p molecule contains 5–7 binding sites for the N-terminal Pex14p fragment. Probably, these binding sites are used one after the other molecule, resulting in a cascade of binding and release during the PTS1 protein import process.

It must be noted that the N-terminus of human Pex14p is protected from externally added protease, suggesting that it is in the peroxisome matrix (Will et al., 1999). This makes an exclusive role for the N-terminus of Pex14p in docking of the Pex5p/cargo complex unlikely. In view of the recent data of Dammai and Subramani (2001) on the recycling of the PTS1 receptor via the peroxisomal lumen, we must face the possibility that Pex14p may also be involved in Pex5p export.

Although it is now generally accepted that Pex14p plays a crucial role in peroxisomal matrix protein import, *H. polymorpha* Pex14p is not essential for this process. We demonstrated that the strong PTS1 protein import defect in an *H. polymorpha* *PEX14* null mutant can be largely restored by overproduction of Pex5p (Salomons et al., 2000). Under these conditions, a major amount of Pex5p accumulated at the outer surface of the peroxisomal membrane, a phenomenon that is never observed in *H. polymorpha* WT cells, not even when Pex5p is overproduced (Van der Klei et al., 1995, 1998). These data suggest that Pex14p is not essential for the initial Pex5p docking. Salomons et al. (2000) argued on the basis of these results that Pex14p might be important for the efficiency of the PTS1 protein import process. However, it must be noted that the suppression of the *pex14* phenotype by Pex5p overproduction only rescued import of AO and DHAS. Import of CAT, eGFP-SKL, eGFP-SKI, eGFP-LARF, and

malate synthase, which also requires Pex5p, was not restored. On the basis of this differential import phenomenon, Kiel and Veenhuis (2000) concluded that apparently some PTS1 proteins are completely dependent on Pex14p whilst others do not require the function of Pex14p to get imported under conditions that sufficient Pex5p is available.

Compared to Pex14p, the interaction of Pex5p with Pex13p seems to be weaker. Using in vitro overlay binding assays, Fransen et al. (1998) were unable to detect direct physical interaction between full-length human Pex5p and Pex13p molecules. However, using a similar technique, *P. pastoris* Pex5p was shown to bind a fragment consisting of the SH3 domain of *P. pastoris* Pex13p (Urquhart et al., 2000). The amount of Pex5p bound to the Pex13p fragment was 20–40 times lower compared to that bound to Pex14p under similar conditions (Urquhart et al., 2000). At first sight, one could conclude that the yeast and human systems differ in the interactions between components of the putative docking site. However, in the experiments by Fransen et al. (1998), the interaction between Pex5p and Pex13p may have been below the level of detection. Alternatively, binding of Pex5p to Pex13p is prevented or reduced when full-length Pex13p is used. This assumption is substantiated by Urquhart et al. (2000), who showed that Pex5p efficiently bound to a fragment containing only the SH3 domain, whereas the interaction was weakened when larger Pex13p fragments were used. On the basis of their data, Urquhart et al. (2000) suggested an alternative role for Pex13p in a later stage of the process that occurs after docking. Based on these Pex5p binding properties, Pex14p seems to be the most likely candidate for recruiting Pex5p at the peroxisomal membrane (Schliebs et al., 1999; Urquhart et al., 2000). However, our recent findings in *H. polymorpha* (Salomons et al., 2000), which suggest that Pex14p is dispensable for Pex5p docking, are inconsistent with this role for Pex14p. Future studies should, therefore, also include the possibility that peroxins that are proposed to be involved in initial Pex5p docking may also function in Pex5p export. These studies should also resolve whether the import site has a dual function and may possibly mediate export as observed for the ER (Plempner and Wolf, 1999).

PTS1 Protein Import: 3. Import Into the Organelle/Release of the Cargo

How matrix proteins actually enter peroxisomes or pre-peroxisomal structures, as described for *Y. lipolytica* (see below), is yet totally unclear. Pex5p has been shown to interact also with the peroxisomal membrane protein Pex12p (Chang et al., 1999; Okumoto et al., 2000) and the matrix protein Pex8p (Rehling et al., 2000). Several authors have proposed that the zinc-binding (RING finger) proteins, Pex10p and Pex12p, eventually together with the third zinc-finger protein Pex2p, may function in the translocation process (Chang et al., 1999, reviewed in Holroyd and Erdmann, 2001). Indeed, Pex12p interacts with Pex10p via their C-terminal zinc binding domains. Furthermore, recently Albertini and coworkers (2001) demonstrated that a RING finger complex is associated with the docking complex. That Pex5p ultimately reaches the

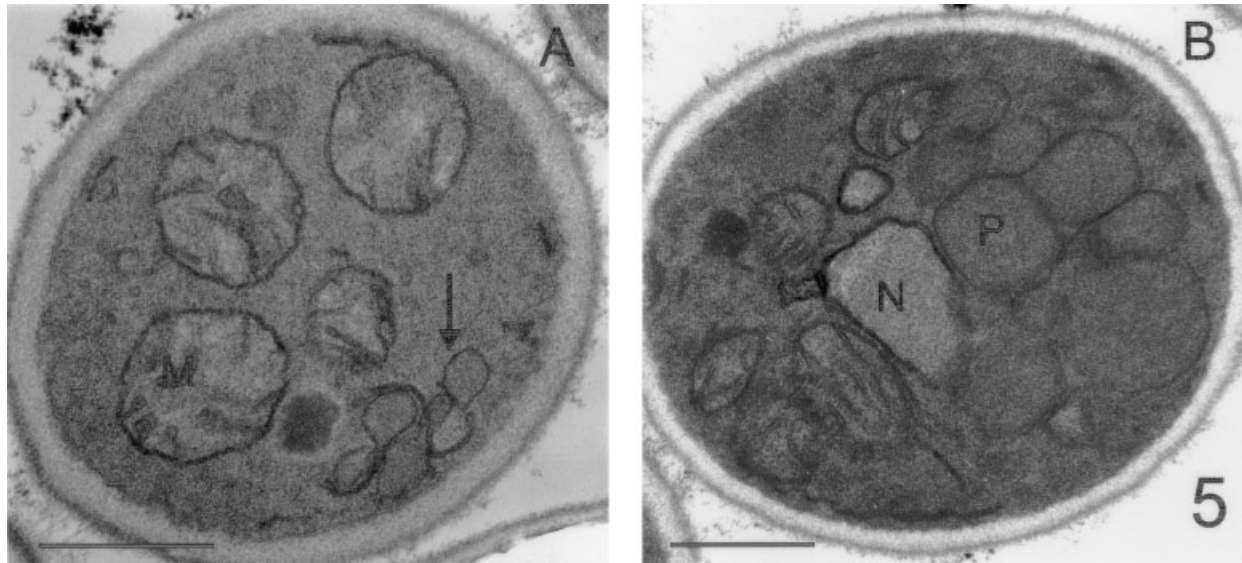


Fig. 5. Restoration of peroxisome formation in *pex4* cell (A) upon overexpression of Pex5p (B). In *pex4* cells, only a few remnants are visualized while the transformant that overproduces Pex5p contains several normal peroxisomes.

interior of the peroxisome has become clear from the elegant studies of Dammai and Subramani (2001).

Pex8p is a remarkable protein; it is the only peroxin that is found in the peroxisomal lumen and it is still unclear why the absence of Pex8p is associated with a *pex* phenotype. *H. polymorpha* Pex8p contains both putative PTS1 and PTS2 signals but both sequences are not required to mediate proper sorting of the protein to the peroxisomal lumen (Waterham et al., 1994; Waterham and Veenhuis, unpublished results). In other species the proteins only contains a PTS1 (Liu et al., 1995; Rehling et al., 2000) or no PTS at all (Smith et al., 1997). Also in baker's yeast the PTS1 was dispensable for its function in matrix protein import. In addition, *S. cerevisiae* Pex8p lacking its PTS1 interacted with the N-terminus of Pex5p (Rehling et al., 2000). These data suggest that Pex8p functions at an intra-organelle stage of the PTS1 protein import cascade. In view of the extended shuttle model, one of the possible options is that Pex8p functions in the release of the cargo protein from the PTS1 receptor (compare Fig. 4).

PTS1 Protein Import: 4. Recycling of Pex5p to the Cytosol

A peroxin that is proposed to be involved in a late stage of Pex5p-dependent protein import is the ubiquitin-conjugating enzyme Pex4p (Crane et al., 1994; Van der Klei et al., 1998; Wiebel and Kunau, 1992). Pex4p faces the cytosol and is associated with the peroxisomal membrane by binding to the integral peroxisomal membrane protein Pex22p (Koller et al., 1999). *H. polymorpha pex4* mutants are not completely blocked in PTS1 protein import (Collins et al., 2000; van der Klei et al., 1998). Comparable to *H. polymorpha pex14*, overproduction of Pex5p also suppressed the PTS1 import defect in *H. polymorpha pex4* cells (Fig. 5). Again a differential import of PTS1 proteins

was observed, in which only AO and DHAS were imported while CAT and MAS were not imported (Kiel and Veenhuis, 2000). Remarkably, in *pex4* cells part of the overproduced Pex5p accumulated at the inner surface of the peroxisomal membrane (Van der Klei et al., 1998). Consistent with the extended shuttle model (Dodt and Gould, 1996; Van der Klei and Veenhuis, 1996), we have interpreted this result in that export/recycling of Pex5p to the cytosol is blocked in the absence of Pex4p. This also readily explains the *pex4* phenotype. The failure to efficiently recycle Pex5p results in the accumulation of part of the protein inside peroxisomes and, thus, in depletion of the cytosolic Pex5p pool and, consequently, a PTS1 protein import defect. In this case, import is dependent on newly synthesized Pex5p and, obviously, overproduction of Pex5p will compensate for the defect and that is exactly what was observed for some PTS1 proteins (AO and DHAS). Apparently, CAT and MAS require additional factors to become imported and that were not overproduced. Since Pex4p is a member of the family of ubiquitin-conjugating enzymes, Pex5p recycling probably involves the modification of a yet unknown protein by ubiquitination.

Also the peroxins Pex1p and Pex6p, members of the AAA family of ATP-ases (see Confalonieri and Duguet 1995), have been implicated in Pex5p recycling (Collins et al., 2000). However, it must be noted that these proteins have been implicated in vesicle fusion processes as well (see below). Furthermore, overexpression of *PEX5* in *H. polymorpha pex1* and *pex6* mutants does not rescue the PTS1 import deficiency (Salomons et al., 2000). Thus, the role of these peroxins in Pex5p recycling may be an indirect one. Alternatively, these proteins have multiple functions.

So far, the evidence suggests that an extended shuttle indeed exists for PTS1 protein import into the peroxisomal matrix. Clearly, the alternative

pathways that have been proposed that do not include import of Pex5p into the peroxisomal matrix have to be reconsidered in view of the recent data of Dammai and Subramani (2001) on Pex5p recycling (Erdmann et al., 1997; Hettema et al., 1999; Olsen, 1998; Subramani, 1998; Urquhart et al., 2000; Waterham and Cregg, 1997). Nevertheless, additional experiments are urgently required to establish whether the human model is generally valid and also operates in yeast.

It must also be noted that *Y. lipolytica* seems to be the major exception to the above extended shuttle mechanism, as Szilard et al. (1995) demonstrated that in this organism Pex5p was exclusively present in the organellar matrix. These authors proposed an alternative function for Pex5p in that it pulls PTS1 proteins across the membrane into the matrix. On the basis of the location of Pex5p in other organisms, this "pulling" model does not seem to reflect a conserved Pex5p function.

Import of PTS2 Containing Matrix Proteins

In fungi, matrix proteins containing a PTS2 sequence are very rare. In baker's yeast only 3-ketoacyl CoA thiolase contains a PTS2 (Glover et al., 1994), while in *H. polymorpha* amine oxidase (AMO) contains this signal also (Faber et al., 1995). The receptor for PTS2 proteins, Pex7p, contains WD40 repeats (Elgersma et al., 1998; Marzioch et al., 1994; Zhang and Lazarow, 1995), which bind the PTS2 signal (Elgersma et al., 1998; Rehling et al., 1996; Zhang and Lazarow, 1996). Initially also the location of Pex7p was controversial. However, the consensus now seems to be that Pex7p is a cycling receptor also. In baker's yeast and *P. pastoris*, Pex7p binds Pex14p at the presumed peroxisomal docking site (Albertini et al., 1997; Johnson et al., 2001). For *S. cerevisiae* it was demonstrated that Pex7p also binds Pex13p (Girzalsky et al., 1999), but not at its SH3 domain. These interactions have also led to the proposal that Pex7p might import matrix proteins via an extended shuttle mechanism. However, it must be noted that for Pex7p no interactions with Pex12p or Pex8p have been reported, although these genes are required for PTS2 import. In *H. polymorpha*, Pex4p does not seem to be required for PTS2 import, because *pex4* mutants still import AMO (van der Klei et al., 1998).

The PTS2 import route utilizes "helper proteins" to import thiolase. In *Y. lipolytica* the mainly cytosolic Pex20p binds thiolase (see below) and thus could make the PTS2 accessible for binding its receptor (Titorenko et al., 1998), although so far no *PEX7* gene has been isolated from *Y. lipolytica*. Interestingly, Pex20p interacts with Pex8p suggesting that it reaches the interior of the peroxisome (Smith and Rachubinski 2001). Also in baker's yeast, the accessory proteins Pex18p and Pex21p are required to import thiolase by binding directly to Pex7p (Purdue et al., 1998). Thus, so far the data concerning PTS2 import are relatively scarce. Clearly additional research is needed to bring the knowledge concerning this route on a par with the PTS1 import route.

PEROXISOMAL MATRIX PROTEIN ASSEMBLY

Little is known of the mechanisms involved in the assembly of peroxisomal matrix proteins. In fact, the subcellular site at which matrix proteins assemble may vary. There is now convincing evidence that matrix proteins may be transported into the organelle in their mature form. Examples of oligomeric matrix protein import have been presented for yeast (Elgersma et al., 1996b; Glover et al., 1994; McNew and Goodman, 1994) and plant (Lee et al., 1997). Hence, the peroxisomal import machinery can accommodate complex, folded proteins. Even import of 9-nm gold particles coated with PTS1-peptides has been reported (Walton et al., 1995). However, this does not mean that folded-protein import is the sole scenario of matrix protein import in peroxisomes. Recently, in *H. polymorpha* we showed that CAT and DHAS can indeed be imported as oligomeric, active enzyme molecules (Faber et al., 2002a). Under the same conditions, AO octamers were not imported. As already noted before, AO is imported as a monomer and is assembled into the active octamer inside the organelle (Evers et al., 1996; Goodman et al., 1984; Waterham et al., 1997). The assembly of the protein is most likely critically dependent on the initial binding of the FAD-cofactor to the AO monomer, a process that surprisingly requires the function of pyruvate carboxylase protein (Ozimek et al., 2003). A schematic representation of our current hypothesis of AO protein import in *H. polymorpha* is shown in Figure 6. The observation that AO is imported as monomers is not unexpected for physiological reasons. AO monomers, even when they contain FAD, are enzymatically inactive (Boteva et al., 1999; Evers et al., 1996). There is a strong need for the cell to prevent premature AO assembly/activation in the cytosol since only minor amounts of active AO in this compartment would give rise to severe energetically disadvantages due to the cytosolic metabolism of hydrogen peroxide and formaldehyde that would retard or even prevent growth on methanol (van der Klei et al., 1991). The requirement of molecular chaperones, as the Hsp70 members in the endoplasmic reticulum (ER) and mitochondria, in peroxisomal matrix protein assembly/import is still matter of debate. Examples do exist that cytosolic Hsp70-type proteins are needed for import in *in vitro* experiments on mammalian (Walton et al., 1994) and plant cells (Crookes and Olsen, 1998). Recently, Harano et al. (2001) reported that in mammalian cells Pex5p recognized its PTS1 cargo by a chaperone-mediated mechanism. Also DnaJ homologues seem to be required in both mammalian and yeast systems (Hettema et al., 1998; Terlecky et al., 1996). However, the precise role of these cytosolic chaperones in matrix protein import in fungi remains to be elucidated.

In yeast *pex* mutants, the assembly of peroxisomal matrix proteins is generally not affected, but normally proceeds in the cytosol (Fig. 7). However, few mutants have been described in which both the import and the assembly of specific enzymes is affected. These include *Y. lipolytica pex20* (Titorenko et al., 1998), a *C. boidinii* mutant lacking the peroxisomal membrane protein Pmp47p (Sakai et al., 1996) and an *H. polymorpha* pyruvate carboxylase (PYC) deficient strain (Ozimek et

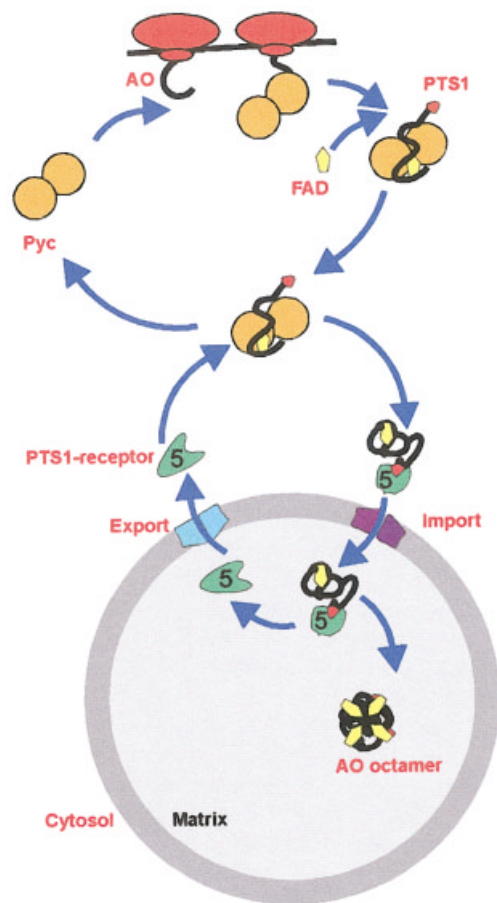


Fig. 6. Hypothetical function of pyruvate carboxylase (pyc) protein in alcohol oxidase (AO) import in peroxisomes. Upon AO synthesis, tetrameric pyc binds to the N-terminus to protect the FAD binding site. After the production of the mature AO molecule, FAD is bound concurrent with the binding of the FAD-containing precursor to Pex5p. In our current view, FAD binding is a prerequisite to allow binding to Pex5p. The subsequent docking and translocation events proceed as depicted in Figure 4. After release from the PTS1 receptor, AO monomers most likely spontaneously assemble in active octamers that are spontaneously arranged in larger crystalloids in the organellar lumen. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

al., 2003). *Y. lipolytica pex20* mutants are selectively blocked in the import and assembly of thiolase. Pex20p is a cytosolic protein that forms a hetero-tetrameric complex with newly synthesized thiolase subunits. Possibly, Pex20p acts as a cytosolic chaperone assisting oligomerisation of thiolase into dimers that normally may occur prior to its import into the peroxisome (Titorenko et al., 1998). *C. boidinii pmp47* mutants mislocalize inactive DHAS molecules to the cytosol. Recent experiments suggest that the role of Pmp47p, a transmembrane protein with similarity to the mitochondrial ADP/ADP carrier, is to import ATP into peroxisomes, which is required for the assembly of DHAS as well as during β -oxidation (Nakagawa et al., 2000; Sakai et al., 1996). In methylotrophic yeasts, PYC is required for the import of AO, but not DHAS or CAT, into peroxisomes (Ozimek et al., 2003). In an *H. poly-*

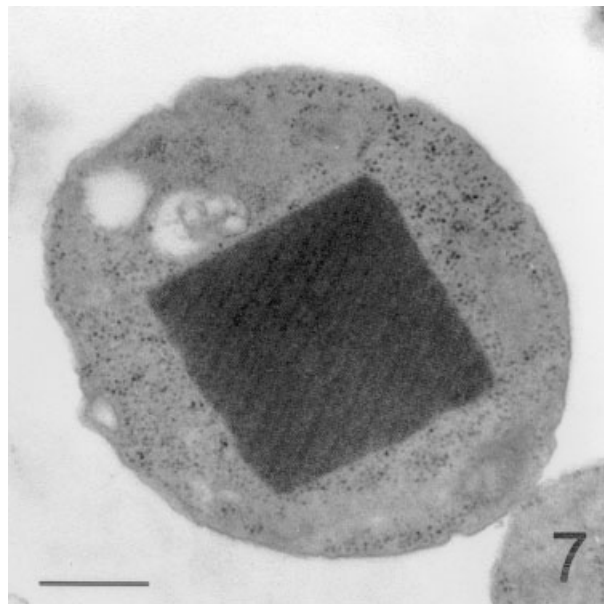


Fig. 7. Section through a protoplast of a *pex* mutant cell, showing the presence of the single large crystal, composed of active octameric AO molecules that are characteristic for such cells.

morpha pyc mutant, AO monomers lacking FAD accumulate in the cytosol, suggesting a role for PYC as a chaperone-like protein that keeps AO competent for FAD binding and, thus, import into peroxisomes.

CAN PEROXISOMES DEVELOP FROM THE ENDOMEMBRANE SYSTEM?

Upon their discovery, peroxisomes were thought to develop by budding from the ER (De Duve and Baudhuin, 1966). Morphological data in yeast provided the first evidence that peroxisomes may multiply by division, a concept that was subsequently substantiated by the finding that peroxisomal proteins are synthesized on free polysomes, followed by post-translational import into peroxisomes (Lazarow and Fujiki, 1985). In yeast, there is solid evidence that this model is true under conditions of normal peroxisome induction. For instance, we unequivocally showed that the small peroxisome present in glucose-grown cells of *H. polymorpha* serves as the template for AO import upon a shift of cells to methanol (Veenhuis et al., 1979). Also, new amine oxidase-containing peroxisomes that develop upon a shift of cells from methanol/methylamine to glucose/methylamine undoubtedly derive from the organelles, originally present in the methanol/methylamine-grown cells (Veenhuis et al., 1981). Indeed, fission of peroxisomes is frequently observed, also by fluorescent methods although the proteins involved in this process (unlike in mitochondrial fission) are not yet known.

Recently, however, data have become available that support alternative modes of peroxisome biogenesis. First, various examples of indirect evidence for a role of the endomembrane system/ER in peroxisome biogenesis were provided (reviewed by Titorenko and Rachubinski, 1998). These include, among others, data from

Salomons et al. (1997) who showed that Brefeldin A interfered with peroxisome biogenesis. Furthermore, the isolation of Pex1p (Erdmann et al., 1991) and Pex6p (Spong and Subramani, 1993; Voorn-Brouwer et al., 1993), both AAA-ATPases that show homology to NSF and Sec18p, proteins that are known to be involved in other membrane fusion processes (Eakle et al., 1988; Wilson et al., 1989), led to suggestions for the occurrence of membrane fusion processes in peroxisome biogenesis. Also, the *Y. lipolytica* glycosylated membrane proteins Pex2p and Pex16p were implicated to reach their target organelle (the peroxisome) via the ER (Titorenko and Rachubinski, 1998). Furthermore, overexpressed ScPex15p was transported to ER-like membranes and became O-glycosylated (Elgersma et al., 1997). Although many of these data pointed to a role for the ER and membrane fusion processes in peroxisome biogenesis, they had to be interpreted with care as, for instance, was exemplified by baker's yeast Pex15p. Although overproduced Pex15p was indeed in part glycosylated and localized in ER-like membranes, these membranes were later on shown to be artificial and to serve as a sink for the excessive amounts of Pex15p (Stroobants, 2001).

More recently, Titorenko and Rachubinski (2001) have provided convincing evidence that in *Y. lipolytica* the development of peroxisomes involves a multi-step process that is initiated by the formation of pre-peroxisomal vesicles that are suggested to arise from a distinct sub-domain of the ER. In their model, these vesicles, termed PPV1 and PPV2, carry distinct subsets of peroxisomal membrane proteins as well as Sec13p and Sec23p, both components of the COPII coat (Titorenko and Rachubinski 1998). These pre-peroxisomal compartments are subsequently converted into the early peroxisomal precursors P1 and P2 by the uptake of additional membrane proteins and release of the COP elements and have now become competent to incorporate distinct sets of matrix proteins. P1 and P2 compartments subsequently fuse in an ATP, Pex1p, and Pex6p dependent process to generate P3 peroxisomes that can mature into normal peroxisomes by a multi-step assembly pathway (Titorenko et al., 2000). How the P1 and P2 vesicles import and accommodate matrix proteins is completely unsolved. However, this fascinating assembly pathway is now accessible to a proteomics approach by the analysis of the various intermediates of the pathway. Undoubtedly, this will lead to the identification of yet unknown components of the matrix protein import machinery. It is relevant to stress here that the above pathway in *Y. lipolytica* deals with the induction of peroxisomes after a shift of cells from peroxisome repressing to peroxisome-inducing conditions. It remains, therefore, to be seen whether during prolonged cultivation of cells the "normal" pathway of growth and division becomes operative or whether peroxisomes in *Y. lipolytica* invariably are formed from precursors and fission does not occur in this organism. In addition, in other organisms comparable mechanisms have not been described yet. It is therefore important to analyze whether the *Y. lipolytica* mechanisms are also operative in other fungi. Indeed, alternative mechanisms for the growth and fission model of peroxisome division seem to exist in yeast. The first example of this was provided by Wa-

terham et al. (1993) who described the re-introduction of peroxisomes in a conditional (Ts) *pex* mutant of *H. polymorpha* that lacked any peroxisomal remnant structures at the restrictive temperature. Later on, comparable observations were made in human cells (South and Gould, 1999). The rapid re-assembly of peroxisomes in these cells lends support to the view that an endomembrane compartment (probably the ER) may have served as an initial template for the formation of novel peroxisomes. How these initial structures are formed remains unsolved. An obvious option is, of course, the mechanism described for *Y. lipolytica*. Recently, we made some observations that lend support for a role of the nuclear membrane in this process (Faber et al., 2002b). An initial indication for this was the observation that re-introduction of the *PEX3* gene in a *pex3* mutant of *H. polymorpha*, which completely lacks peroxisomal membrane remnants, invariably led to the formation of a single small peroxisome in the vicinity of the nuclear membrane (Baerends et al., 1996). We subsequently noticed that synthesis of a fusion protein containing the initial 50 N-terminal amino acids of Pex3p fused to GFP led to the formation of numerous small vesicles that were located at a distinct site of the nuclear membrane. These vesicles lacked peroxisomal matrix proteins but had accumulated peroxisomal membrane proteins. Upon subsequent expression of the full-length *PEX3* gene, a number of these vesicles developed into normal peroxisomes (Faber et al., unpublished data). These observations indicate that a Pex3p receptor moiety must exist at the nuclear membrane. Also, they lend support to the view that these N50-Pex3p-mediated vesicles can be considered pre-peroxisomes that cannot be transformed into normal peroxisomes due to the absence of information residing in the C-terminal part of Pex3p.

Taken together, these data indicate that peroxisome formation may follow different pathways, namely the "classical" way by growth and fission and alternative ways, using the endomembrane system as template. The latter may in particular be important in those cases where peroxisomes are damaged or lost, e.g., due to failure in peroxisome inheritance.

CONCLUDING REMARKS

In the last decade, many of the protein components involved in peroxisome biogenesis (*PEX* genes) have been identified and much learned about their putative function, localization, and possible interaction with other proteins. The present view is that peroxisomes are highly flexible organelles that rapidly assemble and degrade in response to metabolic needs, similar to other components of the endomembrane system as, e.g., the Golgi apparatus. In spite of these achievements, our knowledge of the molecular principles of peroxisome biogenesis and function is hardly beyond the descriptive level. Many questions remain open. For instance, the principles of the matrix protein import machinery are unsolved. This is in part due to the fact that a reliable in vitro yeast matrix protein import assay is lacking. On the other hand, essential components may still be missing. It is, therefore, essential to develop alternative strategies for mutant isolation (e.g., conditional mutants) above the general ones that

are used up to know. Furthermore, biochemical methods that allow isolating functional protein complexes from the peroxisomal membrane may be of value to identify novel, essential components. At present, it is thought that the PTS1 protein import machinery comprises of a cascade of specific interactions (docking of the Pex5p/cargo complex, translocation, recycling of Pex5p) demanding one or more complexes of peroxins in consecutive steps. So far, this view remains only speculative, but novel biophysical techniques (e.g., FRET and FLIM analyses) are now available to solve various protein interactions in detail.

Another intriguing question includes the function of the peroxisomal membrane. To understand the function of the peroxisomal membrane, analysis of its transport properties and the proteins involved is needed. One major problem associated with a biochemical approach to characterize these proteins is that peroxisomal membranes are leaky *in vitro*, probably as a result of the purification procedures. Consequently, it is desirable to set up strategies to clone genes encoding peroxisomal proteins involved in solute transport. As mutants affected in these genes most likely do not have a *pex* phenotype, adapted mutant screens have to be designed or alternatively, transport proteins should be identified by alternative ways (screening of databases, purification of proteins) and their genes cloned by reverse genetics. Analysis of such proteins can subsequently be performed by conventional reconstitution procedures.

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REFERENCES

- Albertini M, Rehling P, Erdmann R, Girzalsky W, Kiel JAKW, Veenhuis M, Kunau WH. 1997. Pex14p, a peroxisomal membrane protein binding both receptors of the two PTS-dependent import pathways. *Cell* 89:83–92.
- Albertini M, Girzalsky W, Veenhuis M, Kunau WH. 2001. Pex12p of *Saccharomyces cerevisiae* is a component of a multi-protein complex essential for peroxisomal matrix protein import. *Eur J Cell Biol* 80:257–270.
- Baerends RJS, Rasmussen SW, Hilbrands RE, van der Heide M, Faber KN, Reuvekamp PT, Kiel JAKW, Cregg JM, van der Klei IJ, Veenhuis M. 1996. The *Hansenula polymorpha* *PER9* gene encodes a peroxisomal membrane protein essential for peroxisome assembly and integrity. *J Biol Chem* 271:8887–8894.
- Barnett P, Bottger G, Klein AT, Tabak HF, Distel B. 2000. The peroxisomal membrane protein Pex13p shows a novel mode of SH3 interaction. *EMBO J* 19:6382–6291.
- Bellu AR, Kiel JAKW. 2003. Selective degradation of peroxisomes in yeasts. *Microsc Res Tech* (in press).
- Bellu AR, Komori M, van der Klei IJ, Kiel JAKW, Veenhuis M. 2001. Peroxisome biogenesis and selective degradation converge at Pex14p. *J Biol Chem* 276:44570–44574.
- Borst P. 1989. Peroxisome biogenesis revisited. *Biochim Biophys Acta* 1008:1–13.
- Boteva R, Visser AJ, Filippi B, Vriend G, Veenhuis M, van der Klei IJ. 1999. Conformational transitions accompanying oligomerization of yeast alcohol oxidase, a peroxisomal flavoenzyme. *Biochemistry* 38:5034–5044.
- Brocard C, Kragler F, Simon MM, Schuster T, Hartig A. 1994. The tetratricopeptide repeat-domain of the PAS10 protein of *Saccharomyces cerevisiae* is essential for binding the peroxisomal targeting signal-SKL. *Biochem Biophys Res Commun* 204:1016–1022.
- Brocard C, Lametschwandtnr G, Koudelka R, Hartig A. 1997. Pex14p is a member of the protein linkage map of Pex5p. *EMBO J* 16:5491–5500.
- Bruinenberg PG, Blaauw M, Kazemier B, AB G. 1990. Cloning and sequencing of the malate synthase gene from *Hansenula polymorpha*. *Yeast* 6:245–254.
- Chang C-C, Warren DS, Sacksteder KA, Gould SJ. 1999. *PEX12* interacts with *PEX5* and *PEX10* and acts downstream of receptor docking in peroxisomal matrix protein import. *J Cell Biol* 147:761–774.
- Collins CS, Kalish JE, Morrell JC, McCaffery JM, Gould SJ. 2000. The peroxisome biogenesis factors pex4p, pex22p, pex1p, and pex6p act in the terminal steps of peroxisomal matrix protein import. *Mol Cell Biol* 20:7516–7526.
- Confalonieri F, Duguet MA. 1995. 200-amino acid ATPase module in search of a basic function. *Bioessays* 17:639–650.
- Crane DI, Kalish JE, Gould SJ. 1994. The *Pichia pastoris* *PAS4* gene encodes a ubiquitin-conjugating enzyme required for peroxisome assembly. *J Biol Chem* 269:21835–21844.
- Crookes WJ, Olsen LJ. 1998. The effects of chaperones and the influence of protein assembly on peroxisomal protein import. *J Biol Chem* 273:17236–17242.
- Dammai V, Subramani S. 2001. The human peroxisomal targeting signal receptor, Pex5p, is translocated into the peroxisomal matrix and recycled to the cytosol. *Cell* 105:187–196.
- De Duve C, Baudhuin P. 1966. Peroxisomes (microbodies and related particles). *Physiol Rev* 46:323–357.
- De Hoop MJ, AB G. 1992. Import of proteins into peroxisomes and other microbodies. *Biochem J* 286:657–669.
- Distel B, Van der Ley I, Veenhuis M, Tabak HF. 1988. Alcohol oxidase expressed under nonmethylotrophic conditions is imported, assembled, and enzymatically active in peroxisomes of *Hansenula polymorpha*. *J Cell Biol* 107:1669–1675.
- Distel B, Erdmann R, Gould SJ, Blobel G, Crane DI, Cregg JM, Dödt G, Fujiki Y, Goodman JM, Just WW, Kiel JAKW, Kunau WH, Lazarow PB, Mannaerts GP, Moser HW, Osumi T, Rachubinski RA, Roscher A, Subramani S, Tabak HF, Tsukamoto T, Valle D, van der Klei I, van Veldhoven PP, Veenhuis M. 1996. A unified nomenclature for peroxisome biogenesis factors. *J Cell Biol* 135:1–3.
- Dödt G, Gould SJ. 1996. Multiple *PEX* genes are required for proper subcellular distribution and stability of Pex5p, the PTS1 receptor: evidence that PTS1 protein import is mediated by a cycling receptor. *J Cell Biol* 135:1763–1774.
- Dödt G, Braverman N, Wong C, Moser A, Moser HW, Watkins P, Valle D, Gould SJ. 1995. Mutations in the PTS1 receptor gene, *PXR1*, define complementation group 2 of the peroxisome biogenesis disorders. *Nat Genet* 9:115–125.
- Eakle KA, Bernstein M, Emr SD. 1988. Characterization of a component of the yeast secretion machinery: identification of the *SEC18* gene product. *Mol Cell Biol* 8:4098–4109.
- Elgersma Y, Kwast L, Klein A, Voorn-Brouwer T, van den Berg M, Metzger B, America T, Tabak HF, Distel B. 1996a. The SH3 domain of the *Saccharomyces cerevisiae* peroxisomal membrane protein Pex13p functions as a docking site for Pex5p, a mobile receptor for the import PTS1-containing proteins. *J Cell Biol* 135:97–109.
- Elgersma Y, Vos A, van den Berg M, van Roermund CW, van der Sluijs P, Distel B, Tabak HF. 1996b. Analysis of the carboxyl-terminal peroxisomal targeting signal 1 in a homologous context in *Saccharomyces cerevisiae*. *J Biol Chem* 271:26375–26382.
- Elgersma Y, Kwast L, van den Berg M, Snyder WB, Distel B, Subramani S, Tabak HF. 1997. Overexpression of Pex15p, a phosphorylated peroxisomal integral membrane protein required for peroxisome assembly in *S. cerevisiae*, causes proliferation of the endoplasmic reticulum membrane. *EMBO J* 16:7326–7341.
- Elgersma Y, Elgersma-Hooisma M, Wenzel T, McCaffery JM, Farquhar MG, Subramani S. 1998. A mobile PTS2 receptor for peroxisomal protein import in *Pichia pastoris*. *J Cell Biol* 140:807–820.
- Erdmann R, Blobel G. 1995. Giant peroxisomes in oleic acid-induced *Saccharomyces cerevisiae* lacking the peroxisomal membrane protein Pmp27p. *J Cell Biol* 128:509–523.
- Erdmann R, Blobel G. 1996. Identification of Pex13p a peroxisomal membrane receptor for the PTS1 recognition factor. *J Cell Biol* 135:111–121.
- Erdmann R, Wiebel FF, Flessau A, Rytka J, Beyer A, Frohlich KU, Kunau WH. 1991. *PAS1*, a yeast gene required for peroxisome biogenesis, encodes a member of a novel family of putative ATPases. *Cell* 64:499–510.
- Erdmann R, Veenhuis M, Kunau WH. 1997. Peroxisomes: organelles at the crossroads. *Trends Cell Biol* 7:400–407.

- Evers ME, Titorenko VI, Harder W, Van der Klei IJ, Veenhuis M. 1996. Flavin adenine dinucleotide binding is the crucial step in alcohol oxidase assembly in the yeast *Hansenula polymorpha*. *Yeast* 12:917–923.
- Faber KN, Keizer-Gunnink I, Pluim D, Harder W, AB G, Veenhuis M. 1995. The N-terminus of amine oxidase of *Hansenula polymorpha* contains a peroxisomal targeting signal. *FEBS Lett* 357:115–120.
- Faber KN, van Dijk R, Keizer-Gunnink I, Koek A, van der Klei IJ, Veenhuis M. 2002a. Import of assembled PTS1 proteins into peroxisomes of the yeast *Hansenula polymorpha*: yes and no! *Biochim Biophys Acta* 1591:157–162.
- Faber KN, Haan GJ, Baerends RJ, Kram AM, Veenhuis M. 2002b. Normal peroxisome development from vesicles induced by truncated *Hansenula polymorpha* Pex3p. *J Biol Chem* 277:11026–11033.
- Fransen M, Brees C, Baumgart E, Vanhooren JC, Baes M, Mannaerts GP, van Veldhoven PP. 1995. Identification and characterization of the putative human peroxisomal C-terminal targeting signal import receptor. *J Biol Chem* 270:7731–7736.
- Fransen M, Terlecky SR, Subramani S. 1998. Identification of a human PTS1 receptor docking protein directly required for peroxisomal protein import. *Proc Natl Acad Sci USA* 95:8087–8092.
- Gatto GJ Jr, Geisbrecht BV, Gould SJ, Berg JM. 2000. Peroxisomal targeting signal-1 recognition by the TPR domains of human PEX5. *Nat Struct Biol* 7:1091–1095.
- Girzalsky W, Rehling P, Stein K, Kipper J, Blank L, Kunau WH, Erdmann R. 1999. Involvement of Pex13p in Pex14p localization and peroxisomal targeting signal 2-dependent protein import into peroxisomes. *J Cell Biol* 144:1151–1162.
- Glover JR, Andrews DW, Rachubinski RA. 1994. *Saccharomyces cerevisiae* peroxisomal thiolase is imported as a dimer. *Proc Natl Acad Sci USA* 91:10541–10545.
- Godecke A, Veenhuis M, Roggenkamp R, Janowicz ZA, Hollenberg CP. 1989. Biosynthesis of the peroxisomal dihydroxyacetone synthase from *Hansenula polymorpha* in *Saccharomyces cerevisiae* induces growth but not proliferation of peroxisomes. *Curr Genet* 16:13–20.
- Goodman JM, Scott CW, Donahue PN, Atherton JP. 1984. Alcohol oxidase assembles post-translationally into the peroxisome of *Candida boidinii*. *J Biol Chem* 259:8485–8493.
- Gould SJ, Keller GA, Hosken N, Wilkinson J, Subramani S. 1989. A conserved tripeptide sorts proteins to peroxisomes. *J Cell Biol* 108:1657–1664.
- Gould SJ, Kalish JE, Morrell JC, Bjorkman J, Urquhart AJ, Crane DI. 1996. Pex13p is an SH3 protein of the peroxisome membrane and a docking factor for the predominantly cytoplasmic PTS1 receptor. *J Cell Biol* 135:85–95.
- Harano T, Nose S, Uezu R, Shimizu N, Fujiki Y. 2001. Hsp70 regulates the interaction between the peroxisome targeting signal type 1 (PTS1)-receptor Pex5p and PTS1. *Biochem J* 357:157–165.
- Hetttema EH, Ruigrok CC, Koerkamp MG, van den Berg M, Tabak HF, Distel B, Braakman I. 1998. The cytosolic DnaJ-like protein djp1p is involved specifically in peroxisomal protein import. *J Cell Biol* 142:421–434.
- Hetttema EH, Distel B, Tabak HF. 1999. Import of proteins into peroxisomes. *Biochim Biophys Acta* 1451:17–34.
- Holroyd C, Erdmann R. 2001. Protein translocation machineries of peroxisomes. *FEBS Lett* 501:6–10.
- Huhse B, Rehling P, Albertini M, Blank L, Meller K, Kunau WH. 1998. Pex17p of *Saccharomyces cerevisiae* is a novel peroxin and component of the peroxisomal protein translocation machinery. *J Cell Biol* 140:49–60.
- Johnson MA, Snyder WB, Cereghino JL, Veenhuis M, Subramani S, Cregg JM. 2001. *Pichia pastoris* Pex14p, a phosphorylated peroxisomal membrane protein, is part of a PTS-receptor docking complex and interacts with many peroxins. *Yeast* 18:621–641.
- Karpichev IV, Small GM. 2000. Evidence for a novel pathway for the targeting of a *Saccharomyces cerevisiae* peroxisomal protein belonging to the isomerase/hydrolase family. *J Cell Sci* 113:533–544.
- Kiel JAKW, Veenhuis M. 2000. Peroxisomal matrix protein import. Suppression of protein import defects in *Hansenula polymorpha* pex mutants by overproduction of the PTS1 receptor Pex5p. *Cell Biochem Biophys* 32:9–19.
- Klein AT, Barnett P, Bottger G, Konings D, Tabak HF, Distel B. 2001. Recognition of peroxisomal targeting signal type 1 by the import receptor Pex5p. *J Biol Chem* 276:15034–15041.
- Koller A, Snyder WB, Faber KN, Wenzel TJ, Rangell L, Keller GA, Subramani S. 1999. Pex22p of *Pichia pastoris*, essential for peroxisomal matrix protein import, anchors the ubiquitin-conjugating enzyme, Pex4p, on the peroxisomal membrane. *J Cell Biol* 146:99–112.
- Komori M, Rasmussen SW, Kiel JAKW, Baerends RJS, Cregg JM, van der Klei IJ, Veenhuis M. 1997. The *Hansenula polymorpha* PEX14 gene encodes a novel peroxisomal membrane protein essential for peroxisome biogenesis. *EMBO J* 16:44–53.
- Komori M, Kiel JAKW, Veenhuis M. 1999. The peroxisomal membrane protein Pex14p of *Hansenula polymorpha* is phosphorylated in vivo. *FEBS Lett* 457:397–399.
- Kragler F, Langer A, Raupachova J, Binder M, Hartig A. 1993. Two independent peroxisomal targeting signals in catalase A of *Saccharomyces cerevisiae*. *J Cell Biol* 120:665–673.
- Lametschwandtner G, Brocard C, Fransen M, van Veldhoven P, Berger J, Hartig A. 1998. The difference in recognition of terminal tripeptides as peroxisomal targeting signal 1 between yeast and human is due to different affinities of their receptor Pex5p to the cognate signal and to residues adjacent to it. *J Biol Chem* 273:33635–33643.
- Lazarow PB, Fujiki Y. 1985. Biogenesis of peroxisomes. *Annu Rev Cell Biol* 1:489–530.
- Lee MS, Mullen RT, Trelease RN. 1997. Oilseed isocitrate lyases lacking their essential type 1 peroxisomal targeting signal are piggybacked to glyoxysomes. *Plant Cell* 9:185–197.
- Liu H, Tan X, Russell KA, Veenhuis M, Cregg JM. 1995. *PER3*, a gene required for peroxisome biogenesis in *Pichia pastoris*, encodes a peroxisomal membrane protein involved in protein import. *J Biol Chem* 270:10940–10951.
- Lorenz P, Maier AG, Baumgart E, Erdmann R, Clayton C. 1998. Elongation and clustering of glycosomes in *Trypanosoma brucei* overexpressing the glycosomal Pex11p. *EMBO J* 17:3542–3555.
- Marshall PA, Krimkevich YI, Lark RH, Dyer JM, Veenhuis M, Goodman JM. 1995. Pmp27 promotes peroxisomal proliferation. *J Cell Biol* 129:345–355.
- Marshall PA, Dyer JM, Quick ME, Goodman JM. 1996. Redox-sensitive homodimerization of Pex11p: a proposed mechanism to regulate peroxisomal division. *J Cell Biol* 135:123–137.
- Marzioch M, Erdmann R, Veenhuis M, Kunau WH. 1994. *PAS7* encodes a novel yeast member of the WD-40 protein family essential for import of 3-oxoacyl-CoA thiolase, a PTS2-containing protein, into peroxisomes. *EMBO J* 13:4908–4918.
- McNew JA, Goodman JM. 1994. An oligomeric protein is imported into peroxisomes in vivo. *J Cell Biol* 127:1245–1257.
- Nakagawa T, Imanaka T, Morita M, Ishiguro K, Yurimoto H, Yamashita A, Kato N, Sakai Y. 2000. Peroxisomal membrane protein Pmp47 is essential in the metabolism of middle-chain fatty acid in yeast peroxisomes and is associated with peroxisome proliferation. *J Biol Chem* 275:3455–3461.
- Okumoto K, Abe I, Fujiki Y. 2000. Molecular anatomy of the peroxin Pex12p: ring finger domain is essential for Pex12p function and interacts with the peroxisome-targeting signal type 1-receptor Pex5p and a ring peroxin, Pex10p. *J Biol Chem* 275:25700–25710.
- Olsen LJ. 1998. The surprising complexity of peroxisome biogenesis. *Plant Mol Biol* 38:163–189.
- Plempner RK, Wolf DH. 1999. Retrograde protein translocation: ERADication of secretory proteins in health and disease. *Trends Biochem Sci* 24:266–70.
- Purdue PE, Yang X, Lazarow PB. 1998. Pex18p and Pex21p, a novel pair of related peroxins essential for peroxisomal targeting by the PTS2 pathway. *J Cell Biol* 143:1859–1869.
- Rachubinski RA, Subramani S. 1995. How proteins penetrate peroxisomes. *Cell* 83:525–528.
- Rehling P, Marzioch M, Niesen F, Wittke E, Veenhuis M, Kunau WH. 1996. The import receptor for the peroxisomal targeting signal 2 (PTS2) in *Saccharomyces cerevisiae* is encoded by the *PAS7* gene. *EMBO J* 15:2901–2913.
- Rehling P, Skaletz-Rorowski A, Girzalsky W, Voorn-Brouwer T, Franse MM, Distel B, Veenhuis M, Kunau WH, Erdmann R. 2000. Pex8p, an intraperoxisomal peroxin of *Saccharomyces cerevisiae* required for protein transport into peroxisomes binds the PTS1 receptor Pex5p. *J Biol Chem* 275:3593–3602.
- Saidowsky J, Dodt G, Kirchberg K, Wegner A, Nastainczyk W, Kunau WH, Schliebs W. 2001. The di-aromatic pentapeptide repeats of the human peroxisome import receptor PEX5 are separate high-affinity binding sites for the peroxisomal membrane protein PEX14. *J Biol Chem* 276:34524–34529.
- Sakai Y, Saiganji A, Yurimoto H, Takabe K, Saiki H, Kato N. 1996. The absence of Pmp47, a putative yeast peroxisomal transporter, causes a defect in transport and folding of a specific matrix enzyme. *J Cell Biol* 134:37–51.
- Salomons FA, van der Klei IJ, Kram AM, Harder W, Veenhuis M. 1997. Brefeldin A interferes with peroxisomal protein sorting in the yeast *Hansenula polymorpha*. *FEBS Lett* 411:133–139.

- Salomons FA, Kiel JAKW, Faber KN, Veenhuis M, van der Klei IJ. 2000. Overproduction of Pex5p stimulates import of alcohol oxidase and dihydroxyacetone synthase in a *Hansenula polymorpha* pex14 null mutant. *J Biol Chem* 275:12603–12611.
- Schliebs W, Saidowsky J, Agianian B, Dödt G, Herberg FW, Kunau WH. 1999. Recombinant human peroxisomal targeting signal receptor PEX5. Structural basis for interaction of PEX5 with PEX14. *J Biol Chem* 274:5666–5673.
- Small GM, Szabo LJ, Lazarow PB. 1988. Acyl-CoA oxidase contains two targeting sequences each of which can mediate protein import into peroxisomes. *EMBO J* 7:1167–1173.
- Smith JJ, Rachubinski RA. 2001. A role for the peroxin Pex8p in Pex20p-dependent thiolase import into peroxisomes of the yeast *Yarrowia lipolytica*. *J Biol Chem* 276:1618–1625.
- Smith JJ, Szilard RK, Marelli M, Rachubinski RA. 1997. The peroxin Pex17p of the yeast *Yarrowia lipolytica* is associated peripherally with the peroxisomal membrane and is required for the import of a subset of matrix proteins. *Mol Cell Biol* 17:2511–2520.
- Snyder WB, Koller A, Choy AJ, Johnson MA, Cregg JM, Rangell L, Keller GA, Subramani S. 1999. Pex17p is required for import of both peroxisome membrane and luminal proteins and interacts with Pex19p and the peroxisome targeting signal-receptor docking complex in *Pichia pastoris*. *Mol Biol Cell* 10:4005–4019.
- South ST, Gould SJ. 1999. Peroxisome synthesis in the absence of preexisting peroxisomes. *J Cell Biol* 144:255–266.
- Spong AP, Subramani S. 1993. Cloning and characterization of PAS5: a gene required for peroxisome biogenesis in the methylotrophic yeast *Pichia pastoris*. *J Cell Biol* 123:535–548.
- Stroobants A. 2001. Studies on peroxisome biogenesis. PhD thesis, University of Amsterdam, The Netherlands.
- Subramani S. 1998. Components involved in peroxisome import, biogenesis, proliferation, turnover, and movement. *Physiol Rev* 78:171–188.
- Swinkels BW, Gould SJ, Bodnar AG, Rachubinski RA, Subramani S. 1991. A novel, cleavable peroxisomal targeting signal at the amino-terminus of the rat 3-ketoacyl-CoA thiolase. *EMBO J* 10:3255–3262.
- Szilard RK, Rachubinski RA. 2000. Tetratricopeptide repeat domain of *Yarrowia lipolytica* Pex5p is essential for recognition of the type 1 peroxisomal targeting signal but does not confer full biological activity on Pex5p. *Biochem J* 346:177–184.
- Szilard RK, Titorenko VI, Veenhuis M, Rachubinski RA. 1995. Pay32p of the yeast *Yarrowia lipolytica* is an intraperoxisomal component of the matrix protein translocation machinery. *J Cell Biol* 131:1453–1469.
- Terlecky SR, Nuttley WM, McCollum D, Sock E, Subramani S. 1995. The *Pichia pastoris* peroxisomal protein PAS8p is the receptor for the C-terminal tripeptide peroxisomal targeting signal. *EMBO J* 14:3627–3634.
- Terlecky SR, Nuttley WM, Subramani S. 1996. The cytosolic and membrane components required for peroxisomal protein import. *Experientia* 52:1050–1054.
- Titorenko VI, Rachubinski RA. 1998. The endoplasmic reticulum plays an essential role in peroxisome biogenesis. *Trends Biochem Sci* 23:231–233.
- Titorenko VI, Rachubinski RA. 2001. Dynamics of peroxisome assembly and function. *Trends Cell Biol* 11:22–29.
- Titorenko VI, Smith JJ, Szilard RK, Rachubinski RA. 1998. Pex20p of the yeast *Yarrowia lipolytica* is required for the oligomerization of thiolase in the cytosol and for its targeting to the peroxisome. *J Cell Biol* 142:403–420.
- Titorenko VI, Chan H, Rachubinski RA. 2000. Fusion of small peroxisomal vesicles in vitro reconstructs an early step in the in vivo multistep peroxisome assembly pathway of *Yarrowia lipolytica*. *J Cell Biol* 148:29–44.
- Urquhart AJ, Kennedy D, Gould SJ, Crane DI. 2000. Interaction of Pex5p, the type 1 peroxisome targeting signal receptor, with the peroxisomal membrane proteins Pex14p and Pex13p. *J Biol Chem* 275:4127–4136.
- Van den Bosch H, Schutgens RB, Wanders RJ, Tager JM. 1992. Biochemistry of peroxisomes. *Annu Rev Biochem* 61:157–197.
- Van der Klei IJ, Veenhuis M. 1996. A molecular analysis of peroxisome biogenesis and function in *Hansenula polymorpha*: a structural and functional analysis. In: Reddy JK, Suga T, Mannaerts GP, Lazarow PB, Subramani S, editors. Peroxisomes: biology and disease. *Ann NY Acad Sci* 804:47–59.
- Van der Klei IJ, Harder W, Veenhuis M. 1991. Methanol metabolism in a peroxisome-deficient mutant of *Hansenula polymorpha*: a physiological study. *Arch Microbiol* 156:15–23.
- Van der Klei IJ, Hilbrands RE, Swaving GJ, Waterham HR, Vrieling EG, Titorenko VI, Cregg JM, Harder W, Veenhuis M. 1995. The *Hansenula polymorpha* PER3 gene is essential for the import of PTS1 proteins into the peroxisomal matrix. *J Biol Chem* 270:17229–17236.
- Van der Klei IJ, Hilbrands RE, Kiel JAKW, Rasmussen SW, Cregg JM, Veenhuis M. 1998. The ubiquitin-conjugating enzyme Pex4p of *Hansenula polymorpha* is required for efficient functioning of the PTS1 import machinery. *EMBO J* 17:3608–3618.
- van Roermund CW, Tabak HF, van Den Berg M, Wanders RJ, Hettema EH. 2000. Pex11p plays a primary role in medium-chain fatty acid oxidation, a process that affects peroxisome number and size in *Saccharomyces cerevisiae*. *J Cell Biol* 150:489–498.
- Veenhuis M, Harder W. 1988. Microbodies in yeasts: structure, function and biogenesis. *Microbiol Sci* 5:347–351.
- Veenhuis M, van Dijken JP, Pilon SA, Harder W. 1978. Development of crystalline peroxisomes in methanol-grown cells of the yeast *Hansenula polymorpha* and its relation to environmental conditions. *Arch Microbiol* 117:153–163.
- Veenhuis M, Keizer I, Harder W. 1979. Characterization of peroxisomes in glucose-grown *Hansenula polymorpha* and their development after the transfer of cells into methanol-containing media. *Arch Microbiol* 120:167–175.
- Veenhuis M, Zwart KB, Harder W. 1981. Biogenesis and turnover of peroxisomes involved in the concurrent oxidation of methanol and methylamine in *Hansenula polymorpha*. *Arch Microbiol* 129:35–41.
- Veenhuis M, Sulter G, van der Klei I, Harder W. 1989. Evidence for functional heterogeneity among microbodies in yeasts. *Arch Microbiol* 151:105–110.
- Voorn-Brouwer T, van der Leij I, Hemrika W, Distel B, Tabak HF. 1993. Sequence of the PAS8 gene, the product of which is essential for biogenesis of peroxisomes in *Saccharomyces cerevisiae*. *Biochim Biophys Acta* 1216:325–328.
- Walton PA, Wendland M, Subramani S, Rachubinski RA, Welch WJ. 1994. Involvement of 70-kD heat-shock proteins in peroxisomal import. *J Cell Biol* 25:1037–1046.
- Walton PA, Hill PE, Subramani S. 1995. Import of stably folded proteins into peroxisomes. *Mol Biol Cell* 6:675–683.
- Waterham HR, Cregg JM. 1997. Peroxisome biogenesis. *Bioessays* 19:57–66.
- Waterham HR, Keizer-Gunnink I, Goodman JM, Harder W, Veenhuis M. 1992a. Development of multipurpose peroxisomes in *Candida boidinii* grown in oleic acid-methanol limited continuous cultures. *J Bacteriol* 174:4057–4063.
- Waterham HR, Titorenko VI, van der Klei IJ, Harder W, Veenhuis M. 1992b. Isolation and characterization of peroxisomal protein import (Pim-) mutants of *Hansenula polymorpha*. *Yeast* 8:961–972.
- Waterham HR, Titorenko VI, Swaving GJ, Harder W, Veenhuis M. 1993. Peroxisomes in the methylotrophic yeast *Hansenula polymorpha* do not necessarily derive from pre-existing organelles. *EMBO J* 12:4785–4794.
- Waterham HR, Titorenko VI, Haima P, Cregg JM, Harder W, Veenhuis M. 1994. The *Hansenula polymorpha* PER1 gene is essential for peroxisome biogenesis and encodes a peroxisomal matrix protein with both carboxy- and amino-terminal targeting signals. *J Cell Biol* 127:737–749.
- Waterham HR, Russell KA, Vries Y, Cregg JM. 1997. Peroxisomal targeting, import, and assembly of alcohol oxidase in *Pichia pastoris*. *J Cell Biol* 139:1419–1431.
- Wiesel FF, Kunau WH. 1992. The Pas2 protein essential for peroxisome biogenesis is related to ubiquitin-conjugating enzymes. *Nature* 359:73–76.
- Wiemer EAC, Nuttley WM, Bertolaet BL, Li X, Francke U, Wheelock MJ, Anne UK, Johnson KR, Subramani S. 1995. Human peroxisomal targeting signal-1 receptor restores peroxisomal protein import in cells from patients with fatal peroxisomal disorders. *J Cell Biol* 130:51–65.
- Will GK, Soukupova M, Hong X, Erdmann KS, Kiel JAKW, Dödt G, Kunau WH, Erdmann R. 1999. Identification and characterization of the human orthologue of yeast Pex14p. *Mol Cell Biol* 19:2265–2277.
- Wilson DW, Wilcox CA, Flynn GC, Chen E, Kuang WJ, Henzel WJ, Block MR, Ullrich A, Rothman JE. 1989. A fusion protein required for vesicle-mediated transport in both mammalian cells and yeast. *Nature* 339:355–359.
- Zhang JW, Lazarow PB. 1995. PEB1 (PAS7) in *Saccharomyces cerevisiae* encodes a hydrophilic, intra-peroxisomal protein that is a member of the WD repeat family and is essential for the import of thiolase into peroxisomes. *J Cell Biol* 129:65–80.
- Zhang JW, Lazarow PB. 1996. Peb1p (Pas7p) is an intraperoxisomal receptor for the NH2-terminal, type 2, peroxisomal targeting sequence of thiolase: Peb1p itself is targeted to peroxisomes by an NH2-terminal peptide. *J Cell Biol* 132:325–334.